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A new marine species of *Euplotes* (Ciliophora, Hypotrichida) from Antarctica

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Synopsis. A new *Euplotes* morphospecies was isolated from marine sand sediments of Terra Nova Bay (Ross Sea, Antarctica), and grown in the laboratory. It belongs to the group of *Euplotes* species which are characterized by a dorsal silver-line system of the 'double' type and a set of 10 frontoventral cirri. A distinctive trait of the new species is a marked polymorphism, which develops in association with a food stimulus. The number of the dorsolateral kineties was found to be normally 10. However, most of the other diagnostic morphological traits showed conspicuous variations: i) the cell body dimensions varied from 38 × 30μm to 110 × 92μm, ii) the number of adoral ciliary membranelles from 45 to 65, iii) the number of silver-stained cortical alveoli aligned in the mid-dorsal interkinetal row from 12 to 18, and iv) the number of kinetosomes of the mid-dorsal kinety from 13 to 22.

INTRODUCTION

The marine Antarctic environment is populated by unexpectedly rich and diverse ciliate communities, which have been found inhabiting sea-ice slush of the lower layer of the pack, open water, sandy bottoms of coastal areas, and tidal pools (Hada, 1961, 1970; Fenchel & Lee, 1972; Thompson, 1972; Thompson & Croom, 1978; Buck & Garrison, 1983; Corliss & Snyder, 1986).

One of the most frequently encountered members of these communities appears to be *Euplotes*, a genus comprising numerous species with a wide geographical distribution and high adaptive potentialities (for a review: Curds, 1975).

Three different Antarctic marine species of *Euplotes* have so far been briefly described on the basis of light microscope observations. One was collected from Weddell Sea, recognized as new, and named *E. antarcticus* by Fenchel & Lee (1972). The other two, not identified, were collected from Palmer Archipelago (Thompson, 1972), and from South Shetland Islands (Thompson & Croom, 1978).

Here we describe, with the aid of the scanning electron microscope, a new Antarctic morphospecies of *Euplotes*, denominated *E. focardii*, three strains of which have been definitively established in our laboratory, where they are maintained under controlled conditions.

The relative ease with which *E. focardii* multiplies under controlled conditions producing large numbers of cells makes it attractive for the investigation of the genetical and biochemical mechanisms by which cell transformations are produced, as well as other relevant aspects of adaptation to the Antarctic environment.

MATERIALS AND METHODS

Sample collection and cell cultures

The sample of seawater and sandy sediment, from which *Euplotes focardii* was isolated, was collected (January, 1988) from a small cove, east of the Italian Antarctic base located at Terra Nova Bay (Ross Sea, 74° 42′ S, 164° 06′ E).

Collection was carried out by means of a 'Petersen' dredge hauled at a depth of 7 m. At the time of collection, the following environmental parameters were recorded: salinity, 35‰; temperature, −1.8° C; pH, 8.1–8.2. The sample was stored in the dark, at 2–4° C, for 3 months before being analyzed.

A dozen unknown *Euplotes*, later assigned to the new species *E. focardii* were individually isolated and supplied either with green algae *Dunaliella tertiolecta*, or unidentified bacteria present in the same antarctic sample and allowed to multiply following suspension with a Luria-Bertani medium (Bacto Tryptone 1%, yeast extract 0.5%).

Three *E. focardii* strains (labelled TN_1, TN_2, and TN_3) were definitively established, two starting from specimens fed with algae and one with bacteria. These strains are currently maintained in a cold room at 2–4° C under a rhythm of 16 h of darkness and 8 h of exposure to very weak light. Mating pair formation was observed neither in unmixed samples of the three strains, nor in their pairwise combinations. All the observations reported in this work were carried out on cells of strain TN_1.

Optical microscopy

Measurements of cell body dimensions refer to specimens fixed with glutaraldehyde vapour and were taken with a calibrated ocular micrometer mounted on an optical microscope. Silver-stained cells were prepared essentially according to Corliss (1953), except that fixation was carried out with 2.5% (v/v) glutaraldehyde in sea water for 60 min, at 4° C. Cells were also prepared according to the pyridinated silver carbonate method of Fernàndez-Galliano (1976).

Scanning electron microscopy

Cells for scanning electron microscopy were fixed for 30 min, at 4° C, using a modified Parducz solution, made by mixing six parts of 2% OsO_4 (w/v) in sea water (instead of in distilled water) and one part of saturated aqueous $HgCl_2$. Fixed cells

were then (1) washed with 0.1 M cacodylate buffer, (2) mounted on cover-slip fragments previously treated with aqueous solution of 0.1% (w/v) L-polylysine, (3) dehydrated in a graded ethanol series, (4) quickly submerged in Freon 113, (5) critical point-dried in a Emscope CPD 750 apparatus, and (6) coated with gold in an Agar Aids sputter coater. Observations were performed with a Stereoscan 200 (Cambridge Instruments Ltd) scanning electron microscope.

Species registration

The holotype slide of silver-stained specimens of *E. focardii* is deposited at the British Museum (Natural History), London; accession number 1989:8:9:1. Cultures of *E. focardii* are available from our laboratory, and one living reference strain (TN_1) has been deposited at the Culture Collection of Algae and Protozoa, the Ferry House, Ambleside, Cumbria LA22 0LP, UK; strain number not yet assigned.

RESULTS

Diagnosis

Marine psammophilic species of *Euplotes* showing a dorsal argyrome of the 'double' type and a marked polymorphism that ensues in cultures suspended with plenty of food. The cirri are: 10 frontoventral, five transverse, and four caudal. Normally kineties (longitudinal rows of bristle cilia) are eight dorsal and two ventrolateral. Body shape may change from ellipsoid to roundish and body dimensions from 38 × 30μm to 110 × 92μm. The mid-dorsal kinety contains 13–22 kinetosomes and is flanked, on the left, by 12–18 polygons of the silver-line system. Adoral ciliary membranelles vary from 45 to 65. Nuclear apparatus consists of a large horseshoe-shaped macronucleus and one small, spherical micronucleus.

Etymology

The species denomination '*focardii*' (from Latin: of Focardi) was chosen in homage to Dr S. Focardi (University of Siena, Italy), who collected the sample of Antarctic water and sand from which *E. focardii* was isolated.

Associated ciliates

Other ciliates were found in association with *E. focardii*. They were identified as belonging to the following genera: *Aspidisca*, *Cyclidium*, *Diophrys*, *Epiclintes*, *Paraurostyla*, *Pleuronema*, *Uronema*, and *Uronychia*.

Morphology

The dorsal pattern of the *Euplotes focardii* argentophilic network (or 'argyrome'), that in silver-stained specimens reveals the juxtaposition of the cortical alveoli, is of 'double' type (Figs 1, 12), according to the designation of *Euplotes* argyrome types proposed by Gates & Curds (1979). Some disruptions of this regular pattern was occasionally observed in the largest specimens.

The number of argyrome polygons (cortical alveoli) that are longitudinally aligned on the left of the mid-dorsal (or central) kinety (identified as the sixth, counting clockwise from the post-oral kinety No. 1) varied from 12 to 18, in relation to the cell body size. On the cell ventral surface, the argentophilic network forms an unsystematic pattern, insignificant for species characterization.

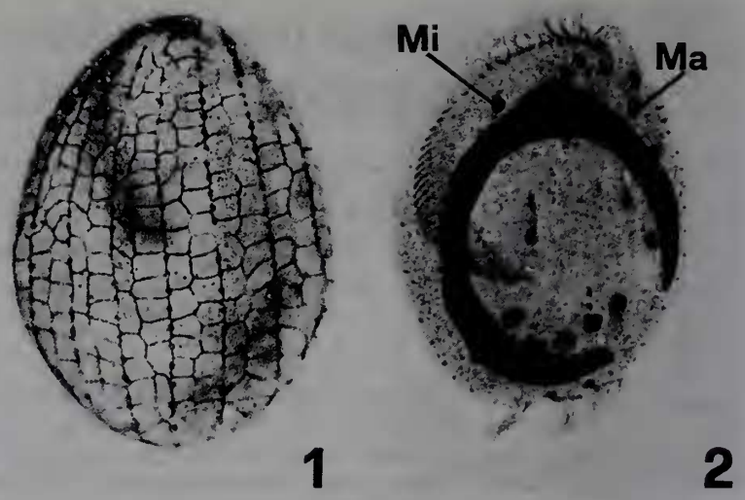


Fig. 1 A silver-stained specimen showing the 'double' pattern of the dorsal argyrome. × 560.

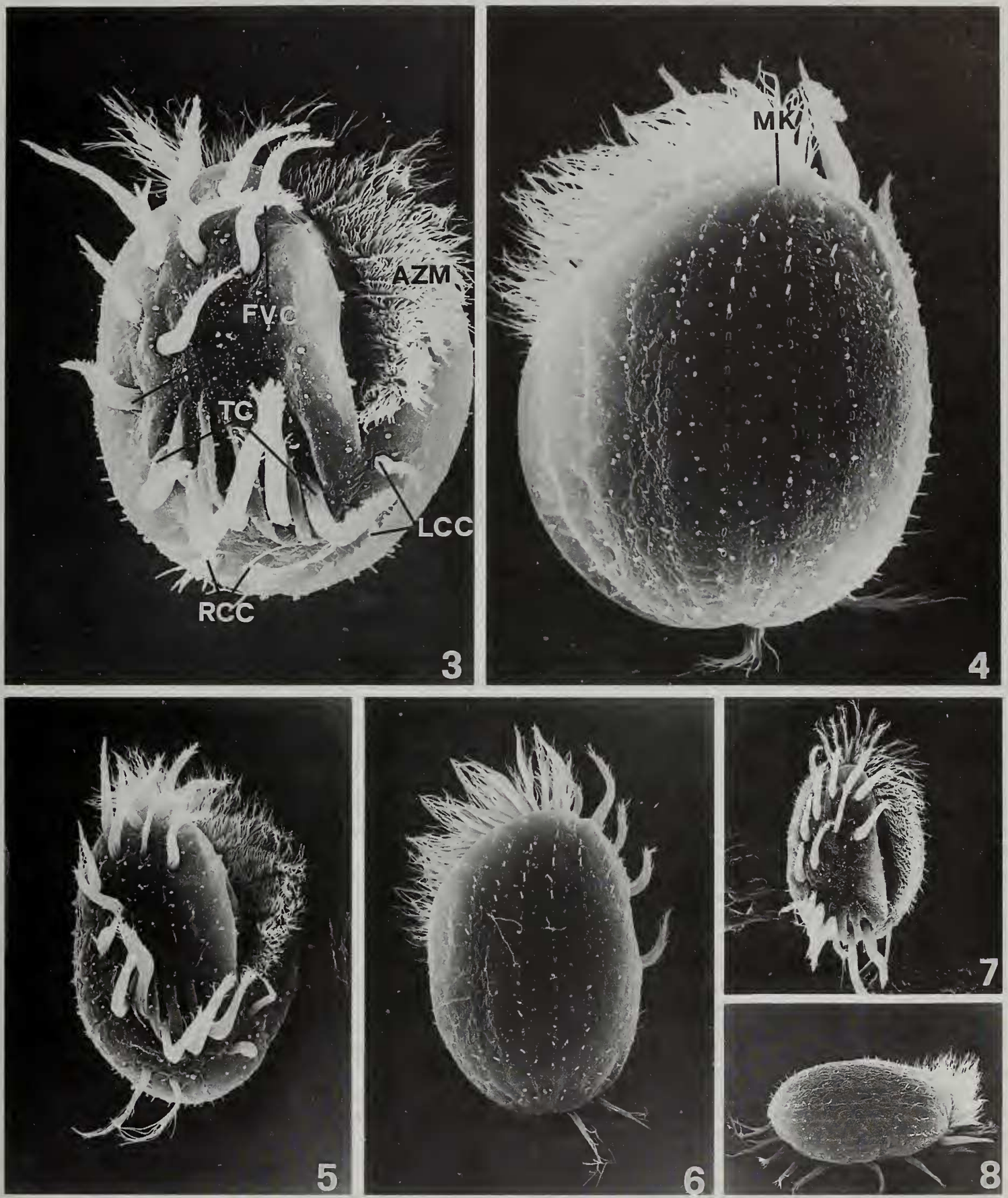
Fig. 2 A specimen in G_1 stage of the cell cycle, stained by Fernàndez–Galiano method, showing the macronucleus (Ma) and the micronucleus (Mi). × 560.

The nuclear apparatus (Fig. 2) consists of one macronucleus, which is horseshoe–shaped and usually huge, and one micronucleus, which is spherical, compact, and usually located close to the macronucleus in the upper left half of the cell.

The contractile vacuole is usually visible in the largest living specimens and located immediately posterior to the insertion sites of those cirri which are conventionally named 'transverse' and labelled (according to Gates, 1977) 1/III and 1/IV.

Euplotes focardii dimensions and shape vary greatly in relation with the cell nutritive conditions, especially when green algae *Dunaliella tertiolecta* were used as food source. Voracious cells that reach 'giant' dimensions of 110 × 92μm regularly develop in cultures in exponential growth phase, when these are suspended with plenty of food (Figs 3, 4). These giant cells, sluggish in movement although very healthy and capable of dividing, usually represent 20–30% of the cell population rather uniformly characterized by average dimensions of 72 × 54μm (Figs 5, 6). Their fission rate is about twice as slow as that of normal cells, whose generation time is in the range of 2–3 days, and the giant feature of the parent cell persists in the two fission products. *Euplotes focardii* may sustain more than 3–4 weeks of starvation without an apparent decrease of viability. The only detectable effect of this prolonged starvation is a sharp reduction of cell body dimension to 40 × 30μm. (Figs 7, 8).

The overall body shape of *E. focardii* is ellipsoidal, with the left margin appreciably more convex than the right. However, cells replete with algae assume a nearly round shape and, in the giant cells, the left side of the body may swell conspicuously to form a transparent, triangular, wing-shaped protuberance. This swelling also demarcates a conspicuous widening of the oral area and of the surrounding adoral zone of membranelles. The anterior border usually appears more convex and shows a definite notch corresponding with the end of the adoral zone of ciliary membranelles. Both the dorsal and ventral surfaces appear poorly sculptured. On the dorsal



Figs 3, 4 Ventral and dorsal view, respectively, of 'giant' specimens isolated from a culture in exponential growth phase and showing the largest dimensions. AZM, adoral zone of membranelles; FVC, frontoventral cirri; LCC, left caudal cirri; MK, mid-dorsal kinety; RCC, right caudal cirri; TC, transverse cirri. × 680.

Figs 5, 6 Ventral and dorsal view, respectively, of morphogenetic stable specimens isolated from a culture at the beginning of the stationary phase, and showing 'standard' dimensions. × 680.

Figs 7, 8 Ventral and dorso-lateral view, respectively, of specimens isolated from a culture exposed to a prolonged starvation (3–4 weeks) and showing the smallest dimensions. × 680.

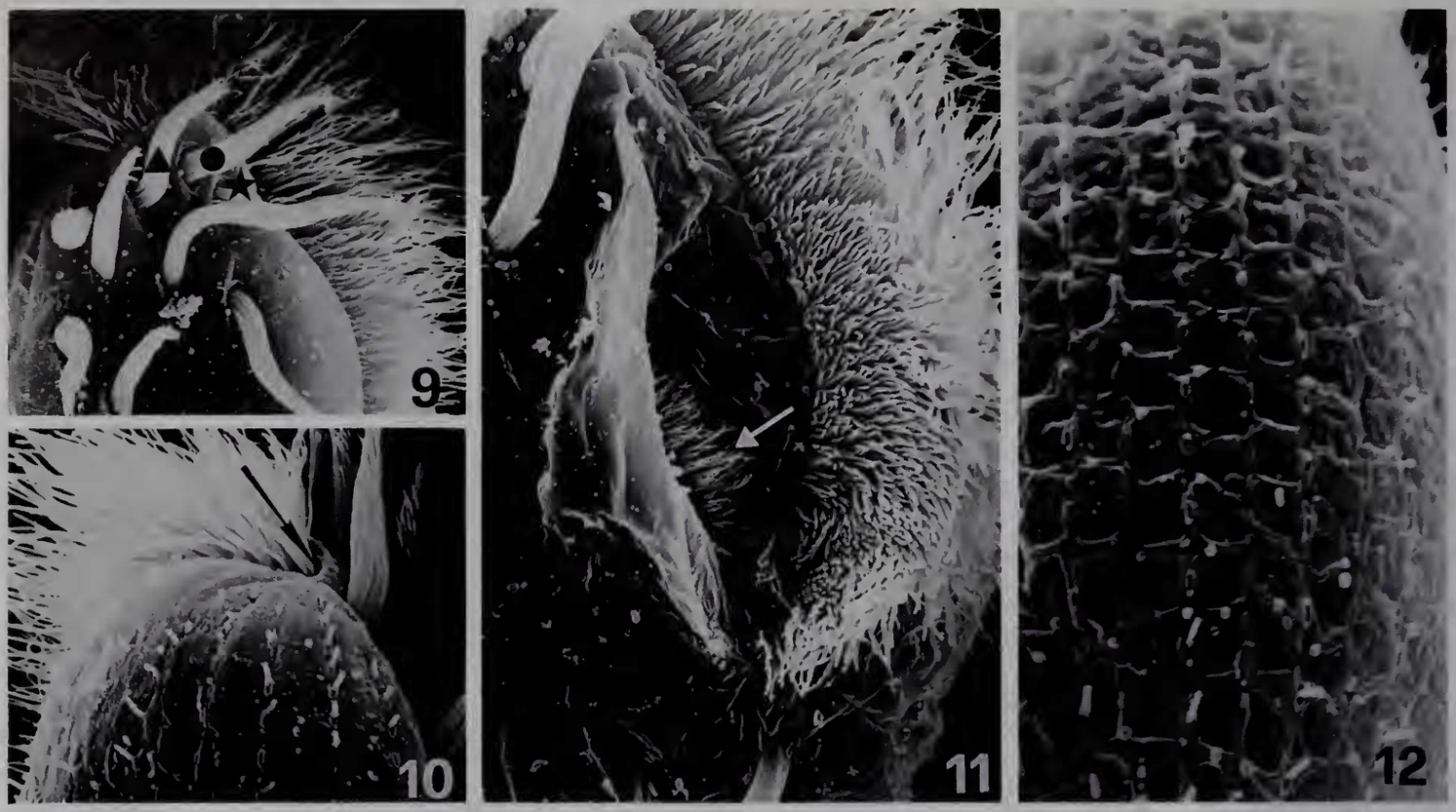


Fig. 9 Anterior region of the ventral cell surface showing the distinctive cone-shaped extremity on which are implanted three frontoventral cirri: 1/0 (star), 3/I (circle), and 3/II (triangle). × 1360.

Fig. 10 Anterior region of the dorsal cell surface showing the groove (indicated by an arrow) where are inserted the anteriormost adoral ciliary membranelles. × 2700.

Fig. 11 Overall view of the cytostomal area showing the well-developed paraoral membrane (indicated by an arrow). × 1700.

Fig. 12 Region of the dorsal cell surface showing, along the mid-dorsal kinety, pits with (arrows) and without (arrowheads) bristles. Also the 'double' pattern of the argyrome is visible. × 1630.

surface, only two ridges running over the left side are rather pronounced; six other ridges are inconspicuous and can be observed only in starved cells. The ventral surface bears four prominent ridges which run for only a short tract anteriorly from the set of five transverse cirri. Anteriorly it assumes a distinctive cone-shaped configuration (Fig. 9), which appears more pronounced in starved cells.

The oral area extends from about one-half of the body length in growing or early stationary-phase cells, up to three-quarters in very starved cells. It is delimited by an inner ribbed margin, which runs nearly straight for most its length. On the outer margin of the oral area are implanted 45–65 adoral membranelles composed of densely packed and unusually long cilia. The membranellar band extensively arches over the anterior border of the cell, where cilia are inserted on the bottom of a groove which deepens over the cone-shaped rostral extension of the ventral cell surface (Fig. 10). The cytostome opens in a rather recessed position and is fringed with a well-developed paraoral membrane (Fig. 11).

Euplotes focardii bears a complex of 19 cirri: 10 are frontoventral, five transverse, and four caudal. Three of the 10 frontoventral cirri—1/0 (or buccal cirrus), 3/I, and 3/II—are typically implanted on the cone-shaped rostral extension. Two of the four caudal cirri are inserted at the very rear of the cell right side and protrude considerably outside the body margin. The other two caudal cirri emerge from the left cell side, with the most anterior one typically positioned immediately below the bottom of the oral area. All cirri appear rather thick and fimbriate apically. This conformation of the cirral distal ends is consistent with the strong thigmotactic behaviour of *E. focardii*.

The bristle cilia align in 10 kineties (counted in samples of at least 100 specimens removed from cultures either in exponential growth, or in stationary phase), eight of which are dorsal and two latero-ventral. In giant specimens, one or two additional kineties, either complete or incomplete, were occasionally observed. It is worth noting that, as observed in scanning electron micrographs of other *Euplotes* species (e.g. Kloetzel, 1975; Ruffolo, 1976; Luporini & Dallai, 1980), cilia are missing in the equatorial and circumequatorial position of the cell, where only empty pits (and not ciliferous kinetosomes) are present.

The kinetosome number of each kinety remarkably varied in cells with different body dimensions. The kinetosomes of the mid-dorsal kinety varied from 13 in the smallest specimens to 22 in the largest.

DISCUSSION

In the genus *Euplotes*, three groups of morphospecies are conventionally distinguished (Gates & Curds, 1979) on the basis of the overall geometrical pattern of the dorsal argyrome—'single', 'double' or 'multiple'—according to the number of longitudinal rows of silver-stained polygons that align between any two adjacent kineties. The species of

Euplotes that we have designated as new, and denominated *E. focardii*, belongs to the group of 33 morphospecies with a dorsal argyrome of the 'double' type.

Within this group, 15 species bear 10 frontoventral cirri and 11 of them—*E. charon* (Müller, 1773), *E. balteatus* (Dujardin, 1841); *E. harpa* Stein, 1859; *E. alatus* Kahl, 1932; *E. trisulcatus* Kahl, 1932; *E. neapolitanus* Wichterman, 1964; *E. octocirratus* Agamaliev, 1967; *E. magnicirratus* Carter, 1972; *E. polycarinatus* Carter, 1972; *E. antarcticus* Fenchel & Lee, 1972; *E. rariseta* Curds, West & Dorahy, 1974—are marine sandwellers like *E. focardii*. Of the other four, three—*E. crenosus* Tuffrau, 1960; *E. inkistans* Tuffrau, 1960; *E. palustris* Ten Hagen, 1980—are inhabitants of fresh-water ponds, and one—*E. tuffraui* Berger, 1965—is endocommensal in the digestive tract of strongylocentroid sea urchins. Among the 11 marine morphospecies with a 'double type' argyrome and cirrotype-10, none shows the following array of structural traits which coexist in *E. focardii*: i) a basic number of 10 kineties; ii) variable numbers of adoral membranelles (45–65), of kinetosomes (13–22) in the mid-dorsal kinety, and of silver-stained polygons (12–18) on the left side of the mid-dorsal kinety; iii) a rostral cone-shaped extension of the cell ventral surface; iv) the leftmost caudal cirrus implanted immediately below the cytostome; v) a huge horseshoe-shaped macronucleus closely associated with a small spherical micronucleus.

This combination of morphological characters makes *E. focardii* clearly distinguishable from the two unidentified Antarctic species of *Euplotes* described by Thompson (1972), which unfortunately cannot be assigned to any group of *Euplotes* morphospecies because their descriptions lack any information on the type of dorsal argyrome.

On the other hand, the morphogenetic capability exhibited by *E. focardii*, in the presence of a persistent abundance of food, to develop giant individuals with an amplified oral area and excess of cortical organelles finds a striking counterpart in only one other marine species of *Euplotes*, *E. balteatus*. In cultures of *E. balteatus*, when the kind of food was experimentally changed from bacteria to small ciliates, Tuffrau (1964) observed the formation of unusally large individuals capable of ingesting more numerous prey. The occurrence of such extensive phenotypic polymorphism in *E. focardii* and *E. balteatus*, jointly with a large convergence of basic morphological traits, may argue for a close evolutionary relationship between these two species. Furthermore, in consideration of the fact that *E. balteatus* manifests a wide geographical distribution and a decidedly extraordinary capacity of exploiting different habitats (e.g., Kahl, 1932; Beers, 1954; Borror, 1963; Berger, 1965), we are led to speculate that *E. focardii* might have evolved by adaptive radiation from this species. Indeed, the capability to adopt different morphotypes in response to changes in the kind and amount of food might have greatly favoured an enhanced survival of individuals of *E. balteatus* spread into the Antarctic environment, where colonizing organisms must be endowed with special devices to cope with sharp seasonal variations in food resources.

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REFERENCES

Agamaliev, F. G. 1967. Faune des ciliés mesopsammiques de la côte ouest de la Mer Caspienne. *Cahiers de Biol. Mar.* **8**: 359–402

Beers, C. D. 1954. *Plagiophyla minuta* and *Euplotes balteatus*, ciliates of the sea urchin *Strongylocentrotus drobachiensis*. *J. Protozool.* **1**: 86–92.

Berger, J. 1965. The infraciliary morphology of *Euplotes tuffraui* n. sp., commensal in Strongylocentrotid echinoids, with comments on echinophilous populations of *Euplotes balteatus* (Dujardin) Ciliata Hypotrichida. *Protistologica* **1**: 17–31.

Borror, A. C. 1963. Morphology and ecology of the benthic ciliated protozoa of Alligator Harbour, Florida. *Arch. Protistenk.* **106**: 465–534.

Buck, K. R. & Garrison, D. L. 1983. Protist from the ice-edge region of the Weddell Sea. *Deep-Sea Res.* **30**: 1261–1277.

Carter, H. P. 1972. Infraciliature of eleven species of the genus *Euplotes*. *Trans. Am. micros. Soc.* **91**: 466–492.

Corliss, J. O. 1953. Silver impregnation of ciliated protozoa by the Chatton–Lwoff technic. *Stain Technol.* **28**: 97–100.

——— **& Snyder, R. A.** 1986. A preliminary description of several new ciliates from the Antarctica, including *Cohnilembus grassei* n.sp. *Protistologica* **22**: 39–46.

Curds, C. R. 1975. A guide to the species of the genus *Euplotes* (Hypotrichida, Ciliatea). *Bull. Br. Mus. nat. Hist.* (Zoology), **28**: 1–61.

———**., West, B. J. & Dorahy, J. E.** 1974. *Euplotes rariseta* sp. n. (Protozoa, Ciliatea), a new small marine hypotrich. *Bull. Br. Mus. nat. Hist.* (Zoology), **27**: 95–102.

Dujardin, F. 1841. *Histoire Naturelle des Zoophytes. Infusoires.* Paris.

Fenchel, T. & Lee, C. C. 1972. Studies on ciliates associated with sea ice from Antarctica. I. The nature of the fauna. *Arch. Protistenk.* **114**: 231–236.

Fernàndez–Galiano, D. 1976. Silver impregnation of ciliated Protozoa: procedure yielding good results with the pyridinated silver carbonate method. *Trans. Am. micros. Soc.* **95**: 557–560.

Gates, M. A. 1977. Analysis of positional information applied to cirral patterns of the ciliate *Euplotes*. *Nature* (N. Y.) **268**: 362–364.

——— **& Curds, C. R.** 1979. The dargyrome of the genus *Euplotes* (Hypotrichida, Ciliophora). *Bull. Br. Mus. nat. Hist.* (Zoology), **35**: 127–200.

Hada, Y. 1961. The pelagic Ciliata from Antarctic waters. *Antarct. Rec.* **11**: 141–145.

——— **1970.** The protozoan plankton of the Antarctic and Subantarctic Seas. *JARE Sci. Rep.* Ser. E **31**: 1–51.

Kahl, A. 1932. Urtiere oder Protozoa. I: Wimpertiere oder Ciliata (Infusoria), eine Bearbietung der freilebenden und ectocommensalen Infusorien der Erde, unter Ausschluss der marinen Tintinnidae. Teil **25**, pp. 399–650. *In*: Dahl, F., *Die Tierwelt Deutschlands*. G. Fischer, Jena.

Kloetzel, J. A. 1975. Scanning electron microscopy of stomatogenesis accompanying conjugation in *Euplotes aediculatus*. *J. Protozool.* **22**: 385–392.

Müller, O. F. 1773. *Vermium Terrestrum et Fluviatilum, seu Animalium Infusorium, Helminthicorum et Testaceorum, non Marinorum, Succincta Historia.* 135 p. Havniae et Lipsiae.

Luporini, P. & Dallai, R. 1980. Sexual interaction of *Euplotes crassus*: differentiation of cellular surfaces in cell-to-cell union. *Develop. Biol.* **77**: 167–177.

Ruffolo, J. J., Jr. 1976. Fine structure of the dorsal bristle complex and pellicle of *Euplotes*. *J. Morph.* **148**: 469–488.

Stein, F. 1859. *Der Organismus der Infusionsthiere nach eingenen Forschungen in systematischer Reihenfolge bearbeiteit.* **206** p.I. Leipzig.

Ten Hagen, R. 1980. A new fresh-water *Euplotes* species: *Euplotes palustris* n.sp. (Ciliophora, Hypotrichida). *Arch. Protistenk.* **123**: 79–83.

Thompson, J. C., Jr. 1972. Ciliated protozoa of the Antarctic Peninsula. pp. 261–288. *In*: Llano, G. A., *Antarctic Terrestrial Biology* (Antarctic Research Series Vol. 20). American Geophysical Union, Washington, D.C.

——— **& Croom, J. M.** 1978. Systematics and ecology of ciliated protozoa from King George Islands. pp. 41–67. *In*: Pawson, D. L., *Biology of the Antarctic Seas VII* (Antarctic Research Series Vol. 27). American Geophysical Union, Washington, D.C.

Tuffrau, M. 1960. Révision du genre *Euplotes*, fondée sur la comparaison des structures superficielles. *Hydrobiologia* **15**: 1–77.

——— 1964. Le maintien des caractères spécifiques à travers le polymorphisme d'*Euplotes balteatus* Dujardin, 1841. *Archs. Zool. exp. gén.* **104**: 143–151.

Wichterman, E. 1964. Description and life cycle of *Euplotes neapolitanus* sp. nov. (Protozoa, Ciliophora, Hypotrichida) from the Gulf of Naples. *Trans. Am. micros. Soc.* **83**: 362–370.

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